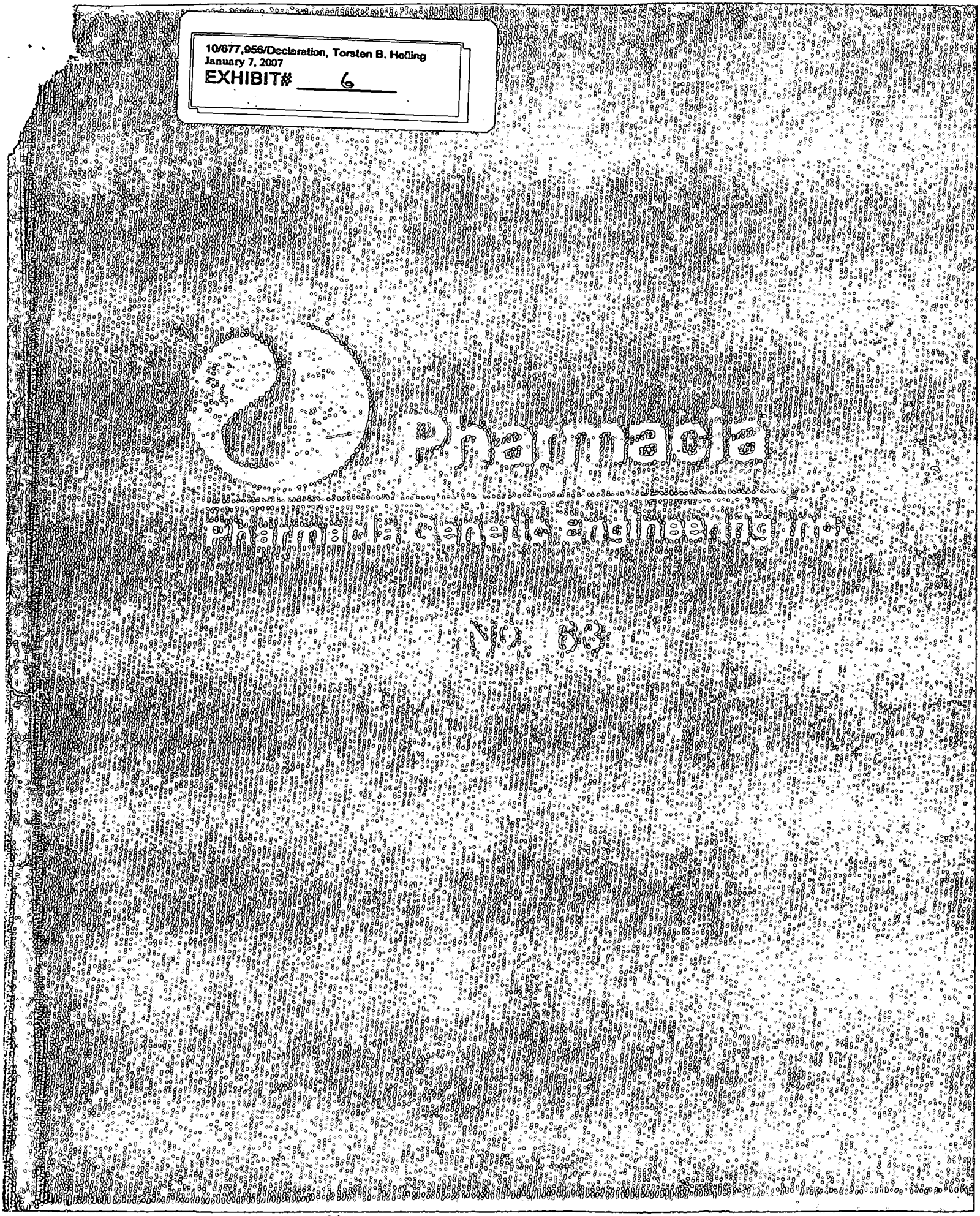
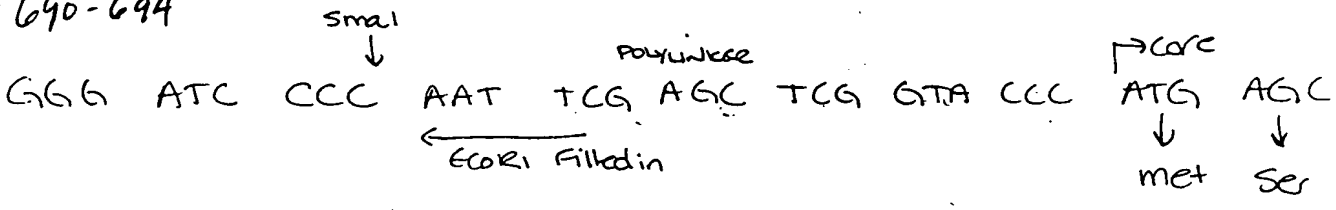
10/677,956/Declaration, Torsten B. Helling
January 7, 2007
EXHIBIT# 6



July 17, 1990 Expression of 690-694 (Cap N)

puc18 690-694 which contains the beginning of the long open reading frame and encodes capsid was cut with EcoRI + BAM and the ends were filled in w/ Klenow. This fragment was gel purified and subsequently ligated into pGEX-3X/SMA the correct orientation will give rise to a 65T/capsid fusion with 6 additional amino acids on the C terminus of 65T. The actual sequence of a plasmid containing the putative correct sequence is shown below

690-694



PGEX 3X vector SmaI cleaved.

insert of 690 691 EcoRI / BamHI cleaved ; filled in

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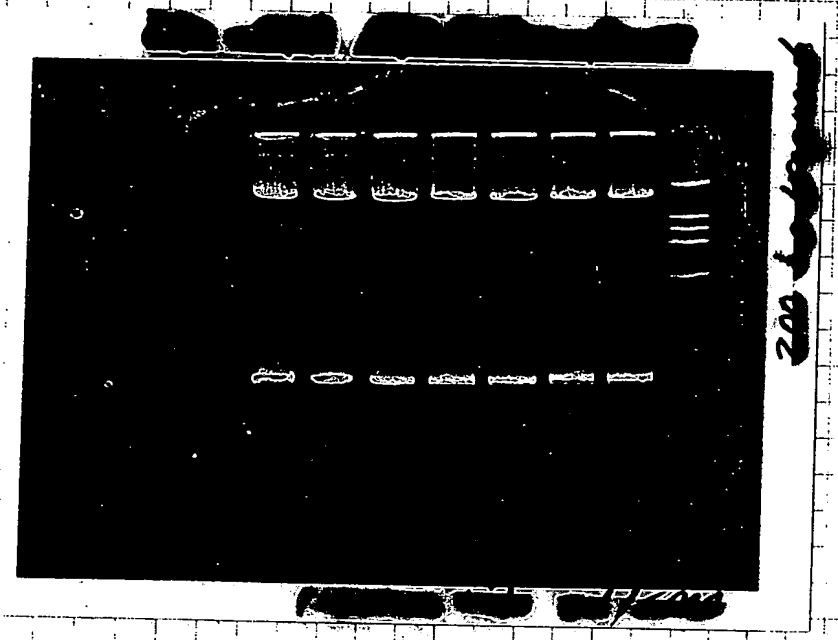
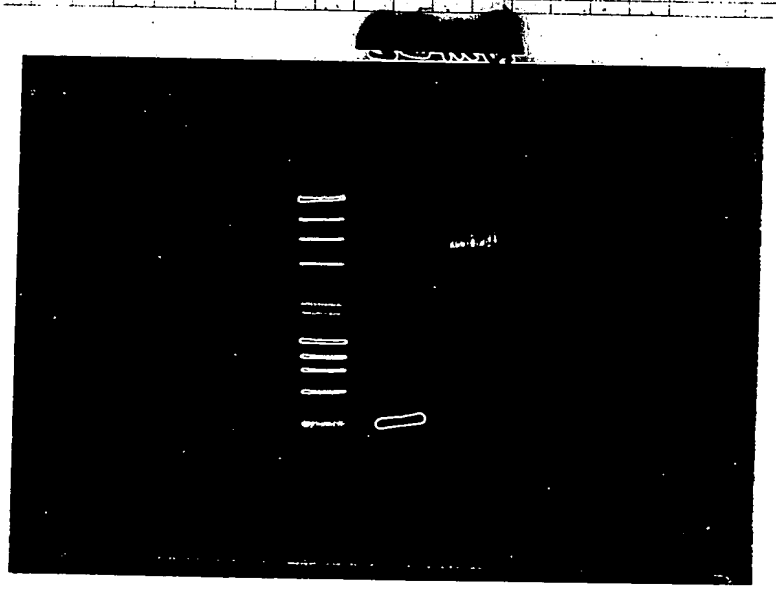
690-694 [Part of insert]

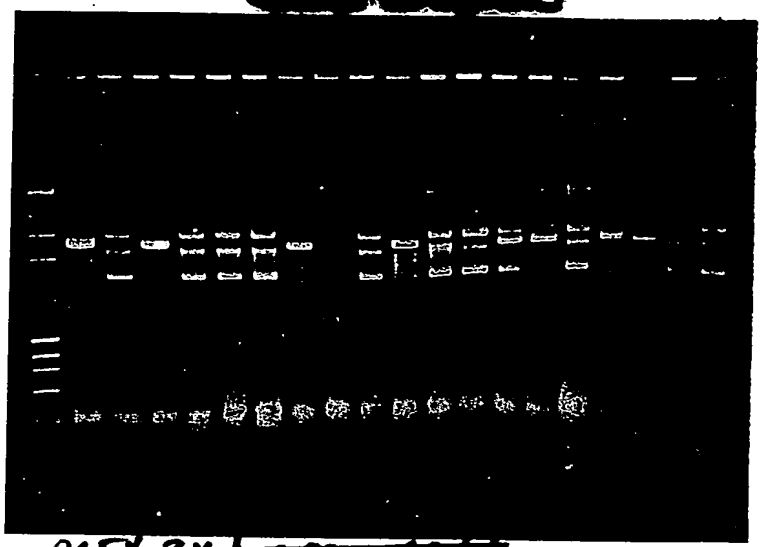
A 5% acryl gel (prep) was run to isolate BAM/RI RI fragment.





A 1% mini gel shows the purified frag. + purified vector.

6 Mini Preps on potential positive 690-694 clones



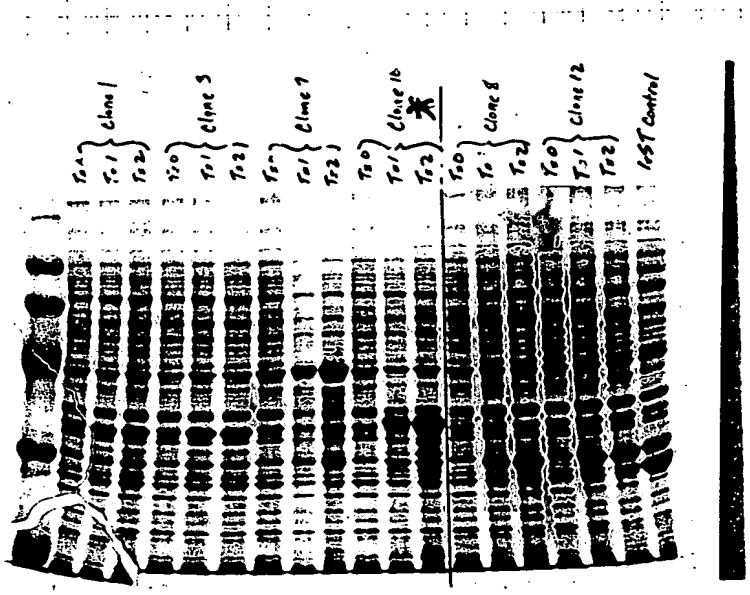
PGEX-2N [redacted]

The insert contains a single Xho I site. Thus, clones 1, 3, 7, 10, 13, 15, 16 are correct in that they contain the 690-694 insert. It will probably be easier to induce them & look for the correct size fusion protein than proceed by standard molecular means to find the correct clone.

Contd. on p. 18

18 July 26-29 Induction of GST/CAPN

6 of the positive mini prep clones were grown o/n, diluted back 1:50 the following day and induced with 1mM IPTG at an OD_{550} of 0.5.



Samples were run on a 12.5% Laemmli gel. Clone 10 appears to be the only positive clone. It makes substantial amts. of the fusion protein. John has broken cells and determined that the majority of protein is insoluble. He tried to purify the soluble fraction without much luck. The insoluble material was solubilized in 8M Guanidine-Hcl and run on an S-300 Column. This material was fractionated and analyzed

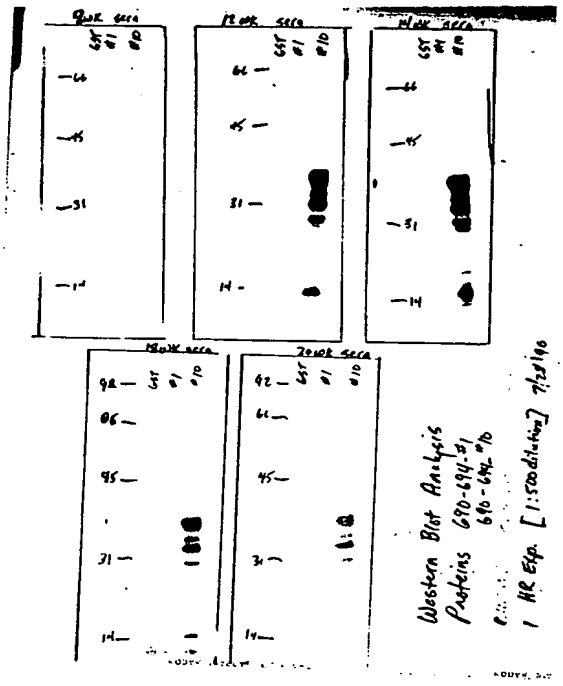
S-300 GST/GAPN

The fractions containing GST/GAPN (35K) were pooled and dialyzed successively into 4M u

containing 10:1 Red: Ox glutathione (3M Ox : 3M Red). A small quantity (10mgs) was saved in 4M. The remaining amt. was refolded into Aqueous by Bob and Audrey.

20. July 29 Western Blot Analysis

Fred + Genevieve are coming soon for a meeting (Monday) so we wanted to test one of the first chimp sera (59) to see if it cross reacts with 690-694.



The results show that CAPN X reacts very well with this chimp sera. Furthermore, there doesn't appear to be any X reactivity with GST or with clone #1 which apparently is negative and acts as a negative control.

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